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Synthesis and transdermal properties of acetylsalicylic acid and selected esters

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Abstract

The primary aim of this study was to determine the transdermal penetration of acetylsalicylic acid and some of its derivatives, to establish a correlation, if any, with selected physicochemical properties and to determine if transdermal application of acetylsalicylic acid and its derivatives will give therapeutic drug concentrations with respect to transdermal flux. Ten derivatives of acetylsalicylic acid were prepared by esterification of acetylsalicyloyl chloride with ten different alcohols. The experimental aqueous solubility, log *D* and transdermal flux values were determined for acetylsalicylic acid and its derivatives at pH 4.5. In vitro penetration was measured through excised female human abdominal skin in diffusion cells. The experimental aqueous solubility of acetylsalicylic acid (6.56 mg/ml) was higher than that of the synthesised acetylsalicylate derivatives (ranging from 1.76×10^{-3} to 3.32 mg/ml), and the log *D* of acetylsalicylic acid (−0.85) was lower than that of its derivatives (ranging from −0.25 to 1.95). There was thus an inverse correlation between the aqueous solubility data and the log *D* values. The experimental transdermal flux of acetylsalicylic acid (263.83 nmol/cm^2 h) was much higher than that of its derivatives (ranging from 0.12 to 136.02 nmol/cm^2 h).
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Keywords: Acetylsalicylic acid; Skin penetration; Transdermal delivery

1. Introduction

The skin is the most extensive and readily accessible organ in the body. In an average adult it covers an area of about 1.73 m^2 and receives one third of circulating blood through the body at any given time. The potential of using intact skin as the site of administration for dermatological preparations to elicit pharmacological action in the skin tissue has been well recognised (Barr, 1962). The permeation of chemicals, toxicants and drugs are much slower across the skin when compared to other biological membranes in the body, due to the excellent barrier provided by the outermost layer of the skin, the stratum corneum (Harrison et al., 1996). The percutaneous delivery of drugs is an effective way of achieving controlled drug delivery. Unfortunately it is only suitable for a limited number of drugs that possesses the appropriate physicochemical characteristics to allow them to cross the stratum corneum (Harrison et al., 1996). The lipophilic stratum corneum is responsible for the primary barrier function of the skin and provides an extensive challenge to scientists in their pursuit to develop drugs for transdermal delivery (Pefile and Smith, 1997).

Acetylsalicylic acid (**1**) possesses anti-inflammatory, analgesic and antipyretic activity. (**1**) is also used in the prevention of thromboembolic disorders, reducing the incidence of colon cancer and it delays the onset of Alzheimer's disease (Insel, 2001; Giovannucci et al., 1995; Rang and Dale, 1999). The most common adverse effect of (**1**) occurring with therapeutic doses is gastro-intestinal disturbances (Reynolds, 1984). Winek et al. (2001) gave the therapeutic blood level of (**1**) for analgesic use as 20–100 μg/ml (0.11–0.56 mM) and the level for prevention of thromboembolic disorders is lower since the dose for these conditions is lower.

Transdermal drug delivery offers a few advantages over oral and parenteral delivery. They include avoiding hepatic first pass metabolism, maintaining constant blood levels for longer periods of time, improving bioavailability, decreasing

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the administered dose, adverse effects and gastrointestinal side effects, easy discontinuation in case of toxic effects and improved patient compliance (Mitragotri, 2000).

The aim of this study was to determine the transdermal penetration of acetylsalicylic acid and some of its derivatives. Nielsen and Bundgaard (1989) evaluated various esters of (**1**) as potential prodrugs and found that (**2**) was not a prodrug for (**1**).

2. Materials and methods

2.1. Materials

Acetylsalicyloyl chloride was purchased from Sigma-Aldrich South Africa Ltd, and HPLC grade acetonitrile was obtained from LabChem South Africa Ltd. All the other reagents and chemicals were of analytical grade.

2.2. General procedures

The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 spectrometer in $CDCl_3$, at a frequency of 300.075 and 75.462 MHz, respectively with tetramethylsilane as internal standard. The MS spectra were recorded on an analytical VG 7070E mass spectrometer using electron impact (EI) at 70 eV as ionisation technique. Melting points were determined by differential scanning calorimetry (DSC) with a Shimadzu DSC-50 instrument.

2.3. High pressure liquid chromatography (HPLC)

The HPLC system consisted of a HP (Hewlet Packard) Agilent 1100 series auto sampler, HP Agilent 1100 series variable wave detector (VWD) and HP Agilent 1100 series isocratic pump. A Phenomenex (Luna C-18, 5 μ, 250 mm × 4.60 mm) column was used together with a Securityguard pre-column (C-18, 4 mm × 3 mm) insert (Phenomenex) in order to prolong column life and the Agilent Chemstation for LC Systems software package was used for data analysis. The flow rate was 1.2 ml/min with detection at 225 nm while the mobile phase compositions and retention times for each of the acetylsalicylic acid derivatives are presented in Table 1. Orthophosphoric acid (OPA) was used to adjust the pH of the mobile phase to 2.25. A volume of 100 μl was injected for each of the samples. Recycling of the mobile phase did not adversely affect the HPLC analysis. Calibration curves were constructed ranging from concentrations of 0.125–5.0 μg/ml.

Table 1
HPLC conditions

Compound	Mobile phase H_2O: acetonitrile	Retention time (min)
Acetylsalicylic acid (**1**)	50:50	3.47
Methyl acetylsalicylate (**2**)	50:50	6.25
Ethyl acetylsalicylate (**3**)	50:50	8.65
Propyl acetylsalicylate (**4**)	33:67	5.66
Isopropyl acetylsalicylate (**5**)	33:67	5.38
n-Butyl acetylsalicylate (**6**)	33:67	7.39
1-Methylpropyl acetylsalicylate (**7**)	33:67	6.84
Tert-butyl acetylsalicylate (**8**)	33:67	6.77
n-Pentyl acetylsalicylate (**9**)	33:67	10.29
1-Methylbutyl acetylsalicylate (**10**)	33:67	9.46
1-Ethylpropyl acetylsalicylate (**11**)	33:67	9.15

2.3.1. Esterification

To a well-stirred mixture of acetylsalicyloyl chloride (5.96 g, 30.0 mmol) in pyridine (1.2 ml, 14.9 mmol) was added one of 10 different alcohols (excess) to form a clear solution. Stirring was continued for 24 h whereafter aqueous HCl (20 ml, 2 M) was added followed by an excess of dichloromethane. The organic phase was collected and washed with 5% $NaHCO_3$ (20 ml) and water (3 × 20 ml). The organic phase was then dried over anhydrous $MgSO_4$, the dichloromethane removed under vacuum and the resulting oil collected. The prepared compounds were purified using column chromatography on silica gel.

*2.3.1.1. Methyl acetylsalicylate (**2**).* A yield of 1.3 g (21.7%) of white crystalline compound was obtained. m.p. 46.80 °C (47–49 °C; Nielsen and Bundgaard, 1989), $C_{10}H_{10}O_4$, ^{1}H NMR δ (ppm) 2.32 (s, 3H, H-3″), 3.85 (s, 3H, H-3′), 7.08 (dd, 1H, J = 8.1, 1.1 Hz, H-6), 7.28 (ddd, 1H, J = 7.6, 7.6, 1.2 Hz, H-4), 7.53 (ddd, 1H, J = 7.4, 7.4, 1.7 Hz, H-5), 8.00 (dd, 1H, J = 7.9, 1.7 Hz, H-3). ^{13}C NMR δ (ppm) 20.90 (C-3″), 52.08 (C-3′), 123.16 (C-2), 123.76 (C-6), 125.92 (C-4), 131.70 (C-3), 133.78 (C-5), 150.68 (C-1), 164.84 (C-1′), 169.57 (C-2″). MS *m*/*z* 194 (14.2), 163 (83.0), 153 (92.5), 120 (52.8), 93 (67.0), 63 (100.0), 43 (49.8).

*2.3.1.2. Ethyl acetylsalicylate (**3**).* A yield of 0.3 g (20.9%) of clear, light orange-yellow oil was obtained, $C_{11}H_{12}O_4$, ^{1}H NMR δ (ppm) 1.34 (t, 3H, J = 7.1 Hz, H-4′), 2.32 (s, 3H, H-3″), 4.31 (q, 2H, J = 7.1 Hz, H-3′), 7.07 (dd, 1H, J = 8.1, 1.1 Hz, H-6), 7.28 (ddd, 1H, J = 7.6, 7.6, 1.2 Hz, H-4), 7.52 (ddd, 1H, J = 7.4, 7.4, 1.7 Hz, H-5), 8.00 (dd, 1H, J = 7.8, 1.8 Hz, H-3). ^{13}C NMR δ (ppm) 14.17 (C-4′), 20.93 (C-3″), 61.02 (C-3′), 123.57 (C-2), 123.69 (C-6), 125.88 (C-4), 131.68 (C-3), 133.60 (C-5), 150.55 (C-1), 164.47 (C-1′), 169.46 (C-2″). MS *m*/*z* 208 (10.3), 163 (67.7), 121 (95.7), 92 (84.4), 65 (63.8), 43 (90.4), 28 (100.0).

*2.3.1.3. n-Propyl acetylsalicylate (**4**).* A yield of 0.6 g (8.4%) of clear, yellow oil was obtained, $C_{12}H_{14}O_4$, ^{1}H NMR δ (ppm) 0.99 (t, 3H, J = 7.4 Hz, H-5′), 1.74 (m, 2H, H-4′), 2.32 (s, 3H, H-3″), 4.21 (t, 2H, J = 6.7 Hz, H-3′), 7.07 (dd, 1H, J = 8.1, 1.1 Hz, H-6), 7.28 (ddd, 1H, J = 7.6, 7.6, 1.2 Hz, H-4), 7.53 (ddd, 1H, J = 7.4, 7.4, 1.7 Hz, H-5), 8.00 (dd, 1H, J = 7.8, 1.7 Hz, H-3). ^{13}C NMR δ (ppm) 10.38 (C-5′), 20.95 (C-3″), 22.00 (C-4′), 66.66 (C-3′), 123.56 (C-2), 123.74 (C-6), 125.90 (C-4), 131.66 (C-3), 133.62 (C-5), 150.63 (C-1), 164.50 (C-1′), 169.52 (C-2″). MS *m*/*z* 222 (12.8), 181 (80.1), 163 (100.0), 65 (98.2), 41 (80.7), 39 (87.3), 27 (87.7).

*2.3.1.4. Isopropyl acetylsalicylate (**5**).* A yield of 0.4 g (6.6%) of clear, dark yellow oil was obtained, $C_{12}H_{14}O_4$, ^{1}H NMR 1.32 (d, 6H, J = 3.7 Hz, H-4′, H-5′), 2.32 (s, 3H, H-3″), 5.20 (m, 1H, J = 6.3 Hz, H-3′), 7.06 (dd, 1H, J = 8.0, 1.1 Hz, H-6), 7.27 (ddd,

1H, *J* = 7.6, 7.6, 1.2 Hz, H-4), 7.51 (ddd, 1H, *J* = 7.4, 7.4, 1.7 Hz, H-5), 7.98 (dd, 1H, *J* = 7.8, 1.7 Hz, H-3). ^{13}C NMR *δ* (ppm) 20.99 (C-3″), 21.82 (C-4′, C-5′), 68.50 (C-3′), 123.63 (C-2), 124.05 (C-6), 125.86 (C-4), 131.64 (C-3), 133.44 (C-5), 150.44 (C-1), 164.06 (C-1′), 169.41 (C-2″). MS *m*/*z* 222 (12.5), 180 (99.0), 163 (79.9), 121 (100.0), 92 (88.0), 65 (68.5), 39 (60.2).

*2.3.1.5. n-Butyl acetylsalicylate (**6**).* A yield of 0.5 g (7.3%) of clear, light orange-yellow oil was obtained, $C_{13}H_{16}O_4$, ^{1}H NMR *δ* (ppm) 0.95 (t, 3H, *J* = 7.4 Hz, H-6′), 1.43 (m, 2H, H-5′), 1.70 (m, 2H, H-4′), 2.32 (s, 3H, H-3″), 4.26 (t, 2H, *J* = 6.7 Hz, H-3′), 7.07 (dd, 1H, *J* = 8.1, 1.1 Hz, H-6), 7.28 (ddd, 1H, *J* = 7.6, 7.6, 1.2 Hz, H-4), 7.53 (ddd, 1H, *J* = 7.4, 7.4, 1.7 Hz, H-5), 7.99 (dd, 1H, *J* = 7.8, 1.7 Hz, H-3). ^{13}C NMR 13.66 (C-6′), 19.15 (C-5′), 20.95 (C-3″), 30.67 (C-4′), 64.94 (C-3′), 123.55 (C-2), 123.74 (C-6), 125.89 (C-4), 131.66 (C-3), 133.61 (C-5), 150.64 (C-1), 164.49 (C-1′), 169.51 (C-2″). MS *m*/*z* 194 (100.0), 138 (96.3), 121 (92.3), 92 (74.2), 43 (86.1), 29 (61.3); FAB 237 ((M + H$^+$) 30.0%), 195 (78.0%), 163 (54.0%), 149 (31.5%), 138 (53.0%), 121 (100.0%).

*2.3.1.6. 1-Methylpropyl acetylsalicylate (**7**).* A yield of 0.6 g (8.3%) of clear, dark yellow oil was obtained, $C_{13}H_{16}O_4$, ^{1}H NMR *δ* (ppm) 0.94 (t, 3H, *J* = 7.4 Hz, H-5′), 1.29 (d, 3H, *J* = 6.3 Hz, H-6′), 1.66 (m, 2H, H-4′), 2.32 (s, 3H, H-3″), 5.04 (m, 1H, H-3′), 7.07 (dd, 1H, *J* = 8.0, 1.0 Hz, H-6), 7.28 (ddd, 1H, *J* = 7.6, 7.6, 1.2 Hz, H-4), 7.51 (ddd, 1H, *J* = 7.4, 7.4, 1.7 Hz, H-5), 7.99 (dd, 1H, *J* = 7.9, 1.7 Hz, H-3). ^{13}C NMR *δ* (ppm) 9.66 (C-5′), 19.42 (C-6′), 21.01 (C-3″), 28.87 (C-4′), 73.06 (C-3′), 123.70 (C-2), 124.05 (C-6), 125.88 (C-4), 131.62 (C-3), 133.45 (C-5), 150.53 (C-1), 164.16 (C-1′), 169.48 (C-2″). MS *m*/*z* 236 (9.3), 194 (89.5), 163 (84.7), 121 (100.0), 92 (84.0), 43 (94.9), 29 (71.3).

*2.3.1.7. Tert-butyl acetylsalicylate (**8**).* A yield of 0.5 g (7.1%) of clear, light orange-yellow oil was obtained, $C_{13}H_{16}O_4$, ^{1}H NMR *δ* (ppm) 1.55 (s, 9H, H-4′, H-5′, H-6′), 2.32 (s, 3H, H-3″), 7.04 (dd, 1H, *J* = 8.1, 1.2 Hz, H-6), 7.26 (ddd, 1H, *J* = 7.6, 7.6, 1.2 Hz, H-4), 7.46 (ddd, 1H, *J* = 7.4, 7.4, 1.8 Hz, H-5), 7.89 (dd, 1H, *J* = 7.8, 1.8 Hz, H-3). ^{13}C NMR *δ* (ppm) 21.08 (C-3″), 28.13 (C-4′, C-5′, C-6′), 81.54 (C-3′), 123.46 (C-2), 125.41 (C-6), 125.77 (C-4), 131.47 (C-3), 132.96 (C-5), 150.12 (C-1), 163.89 (C-1′), 169.39 (C-2″). MS *m*/*z* 181 (60.6), 163 (100.0), 138 (56.5), 121 (71.2), 43 (57.3); FAB 237 ((M + H$^+$) 35.0%), 195 (87.5%), 163 (61.0%), 149 (32.0%), 138 (55.0%), 121 (100.0%).

*2.3.1.8. n-Pentyl acetylsalicylate (**9**).* A yield of 0.6 g (8.0%) of clear, light yellow oil was obtained, $C_{14}H_{18}O_4$, ^{1}H NMR *δ* (ppm) 0.90 (t, 3H, *J* = 7.1 Hz, H-7′), 1.37 (m, 4H, H-5′, H-6′), 1.72 (m, 2H, H-4′), 2.32 (s, 3H, H-3″), 4.25 (t, 2H, *J* = 6.8 Hz, H-3′), 7.07 (dd, 1H, *J* = 8.1, 1.1 Hz, H-6), 7.28 (ddd, 1H, *J* = 7.6, 7.6, 1.2 Hz, H-4), 7.52 (ddd, 1H, *J* = 7.4, 7.4, 1.8 Hz, H-5), 7.99 (dd, 1H, *J* = 7.8, 1.7 Hz, H-3). ^{13}C NMR *δ* (ppm) 13.89 (C-7′), 20.97 (C-3″), 22.30 (C-6′), 28.07 (C-5′), 28.34 (C-4′), 65.25 (C-3′), 123.58 (C-2), 123.75 (C-6), 125.91 (C-4), 131.67 (C-3), 133.61 (C-5), 150.65 (C-1), 164.51 (C-1′), 169.53 (C-2″). MS *m*/*z* 209 (75.1), 208 (94.9), 163 (77.7), 138 (90.2), 121 (86.4), 120 (100.0); FAB 251 ((M + H$^+$) 30.5%), 209 (82.0%), 163 (55.0%), 138 (63.0%), 121 (100.0%).

*2.3.1.9. 1-Methylbutyl acetylsalicylate (**10**).* A yield of 0.2 g (15.5%) of clear, colourless oil was obtained, $C_{14}H_{18}O_4$, ^{1}H NMR *δ* (ppm) 0.92 (t, 3H, *J* = 7.3 Hz, H-6′), 1.30 (d, 3H, *J* = 6.3 Hz, H-7′), 1.38 (m, 2H, H-5′), 1.60 (m, 2H, H-4′), 2.32 (s, 3H, H-3″), 5.12 (m, 1H, H-3′), 7.07 (dd, 1H, *J* = 8.1, 1.1 Hz, H-6), 7.28 (ddd, 1H, *J* = 7.6, 7.6, 1.2 Hz, H-4), 7.51 (ddd, 1H, *J* = 7.4, 7.4, 1.8 Hz, H-5), 7.98 (dd, 1H, *J* = 7.8, 1.7 Hz, H-3). ^{13}C NMR *δ* (ppm) 13.88 (C-6′), 18.62 (C-7′), 19.95 (C-5′), 21.01 (C-3″), 38.15 (C-4′), 71.65 (C-3′), 123.70 (C-2), 124.06 (C-6), 125.88 (C-4), 131.62 (C-3), 133.44 (C-5), 150.53 (C-1), 164.14 (C-1′), 169.46 (C-2″). MS *m*/*z* 250 (7.9), 208 (88.4), 163 (83.1), 139 (58.1), 121 (100.0), 92 (80.9), 65 (58.2).

*2.3.1.10. 1-Ethylpropyl acetylsalicylate (**11**).* A yield of 0.2 g (10.9%) of clear, colourless oil was obtained, $C_{14}H_{18}O_4$, ^{1}H NMR *δ* (ppm) 0.92 (t, 6H, *J* = 7.5 Hz, H-5′, H-7′), 1.65 (m, 4H, H-4′, H-6′), 2.32 (s, 3H, H-3″), 4.96 (m, 1H, H-3′), 7.07 (dd, 1H, *J* = 8.1, 1.1 Hz, H-6), 7.28 (ddd, 1H, *J* = 7.6, 7.6, 1.2 Hz, H-4), 7.52 (ddd, 1H, *J* = 7.4, 7.4, 1.8 Hz, H-5), 8.00 (dd, 1H, *J* = 7.8, 1.7 Hz, H-3). ^{13}C NMR *δ* (ppm) 9.56 (C-5′, C-7′), 20.99 (C-3″), 26.47 (C-4′, C-6′), 77.54 (C-3′), 123.75 (C-2), 124.02 (C-6), 125.88 (C-4), 131.59 (C-3), 133.44 (C-5), 150.60 (C-1), 164.33 (C-1′), 169.47 (C-2″). MS *m*/*z* 208 (80.7), 163 (94.2), 138 (98.3), 121 (100.0), 92 (74.3), 43 (63.4); FAB 251 ((M + H$^+$) 23.0%), 209 (10.0%), 181 (16.5%), 163 (35.0%), 139 (21.0%), 121 (100.0%).

2.4. Physicochemical properties

2.4.1. Solubility determination

The aqueous solubility of acetylsalicylic acid and its derivatives was obtained by preparing saturated solutions in a tris-(hydroxymethyl)aminomethane buffer (TRIS) at pH 4.5. The slurries were stirred with magnetic bars in a water bath at 37 °C for 24 h. An excess of solute (oil or crystals) was present at all times to provide saturated solutions. After 24 h, the solutions were filtered and analysed directly by HPLC to determine the concentration of solute dissolved in the solvent. The experiment was done in triplicate.

2.4.2. Experimental log D

Equal volumes of *n*-octanol and TRIS buffer (pH 4.5) were saturated with each other under vigorous stirring for at least 24 h. Each of the acetylsalicylic acid derivatives (10 mg/ml) was dissolved in 3 ml pre-saturated *n*-octanol, stoppered and agitated for 10 min. Subsequently 3 ml pre-saturated TRIS buffer was transferred to assay tubes containing before-mentioned solutions. The tubes were stoppered and agitated for 45 min. Thereafter, they were centrifuged at 4000 rpm for 30 min. The *n*-octanol and aqueous phases were separately analysed by HPLC. The aqueous phase was analysed directly by HPLC and the *n*-octanol solutions were diluted 1:1000 with acetonitrile (solvent) prior to being analysed by HPLC. The log *D* values (log (octanol: pH 4.5 buffer partition coefficient)) were calculated as logarithmic

ratios of the acetylsalicylic acid derivative concentrations in the *n*-octanol phase to the concentrations in the TRIS buffer. The experiment was done in triplicate.

2.5. Permeation experiments

2.5.1. Preparation of donor solutions

Donor solutions of the acetylsalicylate derivatives were obtained by the equilibration of excess amounts of solute in TRIS buffer (pH 4.5). TRIS buffer was used because over the period of the experiment the derivatives were stable in it, while in phosphate buffer solution (PBS) the derivatives were absent after 24 h. The slurries were prepared in stoppered flasks with stirring in a water bath at 37 °C over a period of 24 h solvent saturation to occur. An excess amount of solute was present at all times.

2.5.2. Skin preparation

Female human abdominal skin, obtained after cosmetic procedures, was used for the permeation studies. A scalpel was used to separate the skin from the fat layer; subsequently the epidermis was removed by means of immersion in 60 °C HPLC water for 60 s (Kligman and Christophers, 1963).

The epidermis was gently teased away from the skin with forceps. Special care was taken that the integrity of the epidermis was not ruptured, as this would compromise the validity of the results. The epidermis was placed in a bath filled with HPLC water and carefully set on Whatman® filter paper, left to air dry and was wrapped in foil. The foil containing the epidermis was stored in a freezer at −20 °C and was used within 6 months after being prepared. Prior to use, the epidermis was thawed and visually examined, before it was mounted on the Franz diffusion cells.

2.5.3. Method for skin permeation

Vertical Franz diffusion cells with 2.0 ml receptor compartments and 1.0751 cm^2 effective diffusion area was used for the permeation studies. The epidermal skin layer was carefully mounted on the lower half of the Franz cell with the stratum corneum facing upwards. A clamp was used to fasten the upper and lower parts of the Franz cell together, with the epidermis separating the donor and receptor compartments. The receptor compartments were filled with isotonic TRIS buffer (pH 7.4). Special care was taken that no air bubbles came between the buffer solution and the epidermis. The Franz cells, containing buffer solution, were equilibrated for 1 h in the water bath at 37 °C, prior to the addition of the saturated solutions to the donor compartments. Only the receptor compartments were submerged in the water and were equipped with stirring magnets. After a period of 1 h, 1.0 ml of freshly prepared saturated solution with an excess oil or crystals were added to each donor compartment, which was immediately covered with Parafilm® to prevent the evaporation of any constituents from the saturated solution for the duration of the experiment. An excess amount of solute was present in the donor compartments at all times during the experimental procedure.

The entire receptor volumes were withdrawn and replaced with 37 °C fresh buffer solution (pH 7.4) after 2, 4, 6, 8, 10, 12 and 24 h. The entire receptor volumes were withdrawn to mimic sink conditions as they occur in the human body. The experiments were conducted over 24-h periods.

The withdrawn samples were assayed immediately by HPLC to in each case determine the drug concentration of (**1**) or the derivative (**2**)–(**11**) that had permeated the epidermis. In no case any salicylic acid or (**1**) was observed in the chromatograms of the receptor phase of the derivatives in the TRIS buffer, although (**2**)–(**11**) showed an extra peak at 1.790–2.170 min in the chromatogram. The experiment was done until six values within 30–40% of one another were obtained. Flux was calculated from the gradient of the cumulative concentration versus time graph. At least six data points on the steady-state part of the curve were used.

3. Results

Table 2.

4. Discussion

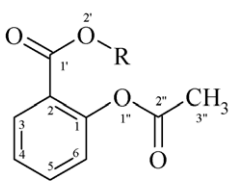
4.1. Structures of the products

4.1.1. 1-Methylbutyl acetylsalicylate (*10*)

The MS data (FAB) confirmed the presence of the molecular ion of (**10**) at *m*/*z* 250, corresponding to a molecular formula of $C_{14}H_{18}O_4$. The molecular ion was not observed in the electron

Table 2
Aqueous solubility, partition coefficient and flux of acetylsalicylic acid and its derivatives

Compound	Aqueous solubility (mg/ml)	Aqueous solubility (mM)	log *D*	Flux (μg/cm^2 h)	Flux (nmol/cm^2 h)
Acetylsalicylic acid (**1**)	6.56	36.41	−0.85	47.53 ± 5.8	263.83
Methyl acetylsalicylate (**2**)	3.26	16.79	−0.25	10.06 ± 3.4	51.81
Ethyl acetylsalicylate (**3**)	3.32	15.95	0.30	28.32 ± 8.0	136.02
Propyl acetylsalicylate (**4**)	1.60	7.20	0.86	1.79 ± 0.6	8.05
Isopropyl acetylsalicylate (**5**)	0.52	2.34	0.75	2.35 ± 0.6	10.57
n-Butyl acetylsalicylate (**6**)	0.07	0.30	1.32	1.59 ± 0.3	6.73
1-Methylpropyl acetylsalicylate (**7**)	0.20	0.85	1.23	1.69 ± 0.4	7.15
Tert-butyl acetylsalicylate (**8**)	0.76	3.22	1.09	7.30 ± 1.0	30.90
n-Pentyl acetylsalicylate (**9**)	1.76×10^{-3}	0.01	1.95	0.03 ± 0.0	0.12
1-Methylbutyl acetylsalicylate (**10**)	0.05	0.20	1.64	0.05 ± 0.03	0.20
1-Ethylpropyl acetylsalicylate (**11**)	0.11	0.44	1.65	0.34 ± 0.1	1.36



1: R = H
2: R = CH_3
3: R = C_2H_5
4, 5: R = *n*-C_3H_7, *i*-C_3H_7
6, 7, 8: R = *n*-C_4H_9, 2°-C_4H_9, *t*-C_4H_9
9, 10: R = *n*-C_5H_{11}, 2°-C_5H_{11}
11: R = $CH(C_2H_5)_2$

Fig. 1. Structures of acetylsalicylic acid and its esters.

impact mass spectrum. The ^{13}C NMR data of (**10**) were similar to that of (**1**), except that the signal of the carbonyl carbon atom has shifted to a slightly lower magnetic field at δ 164.14 in the ^{13}C NMR spectrum, while the presence of the methyl groups were indicated by signals at δ 13.88 and 18.62, with the methine group represented by the peak at δ 71.65. The methylene groups were indicated by signals at δ 19.95 and 38.15. In the ^{1}H NMR spectrum the triplet at δ 0.92 and the doublet at δ 1.30 represent two methyl groups. The multiplets at δ 1.38 and 1.60 represent two methylene groups, while the signal at δ 5.12 represents the methine group. The structural properties of the remaining derivatives were consistent with that of (**10**) and the structures of all the derivatives (Fig. 1) were determined in the same fashion.

4.2. Physicochemical properties

A higher degree of ionisation leads to lower concentrations of unionised drug available for transdermal penetration. Hence, unionised species permeate the stratum corneum better than the ionised form (Abdou, 1989; Jack et al., 1991). (**1**) is a relatively strong acidic drug with a pKa of 3.50 (Florey, 1979), thus at a pH of 3.5 (**1**) is 50% unionised. This provides a problem for transdermal application, for if the pH is lower than 4.5 it will irritate the skin. This was the motive for choosing a pH of 4.5, where (**1**) was 9.09% unionised. To compare the synthesised derivatives with (**1**), it was necessary to determine the physiocochemical properties at the same pH.

The aqueous solubility of (**1**) is higher and the log *D* of (**1**) is lower than that of the synthesised acetylsalicylate compounds. Table 2 shows that in the straight chain series (**2**), (**3**), (**4**), (**6**) and (**9**) the aqueous solubility on a molar basis decreases and the log *D* increases with an increase in alkyl chain length, which is in accordance with the findings of Abdou (1989). The effect of branching in the promoiety leads to an increase in aqueous solubility and a decrease in log *D* values in the series (**6**)–(**8**) and (**9**)–(**11**), but not for (**4**) and (**5**). These results are in agreement with predictions in the literature (Sloan et al., 2003).

The results indicate that esterification of (**1**) leads to lower aqueous solubilities and higher log *D* values. These results validate the aqueous solubility data, whereby compounds with higher log *D* values present with an increased lipophilicity and lower aqueous solubility.

4.3. Transdermal properties

The experimental transdermal flux of (**1**) (47.53 $\mu g/cm^2$ h) was much higher than that of its derivatives, with the (**3**) (28.32 $\mu g/cm^2$ h), (**2**) (10.06 $\mu g/cm^2$ h) and (**8**) derivatives (7.30 $\mu g/cm^2$ h) being the only compounds with appreciable flux. (**1**) and (**2**) were both crystalline compounds, the rest of the acetylsalicylate derivatives were oils. Therefore, in the supersaturated buffer solutions (**1**) and (**2**) had crystals at the bottom of the donor phase, on top of the skin, keeping the resultant solution homogenous which may explain the higher flux observed for these compounds. The other derivatives were found floating on top of the saturated solution, and as there was no stirring in the donor phase to keep the resultant solution homogenous, the solution closest to the skin became unsaturated and this may describe the decreased flux observed for these compounds, except for (**3**). If solution of the compound from the oil phase is slow, it is possible that the concentration immediately adjacent to the skin surface will not be replenish faster than it is absorbed into the skin. In this case there will be a depletion of the concentration that drives the flux.

As a general rule, a drug substance with an aqueous solubility of less than 1 mg/ml may represent a potential bioavailability problem. A drug should have optimal permeation and highest flux values if it has reasonable solubility in both water and oils and has a log *D* in the range of 1–2 (Sloan, 1989; Hadgraft, 1996; Roberts and Sloan, 2000). (**1**), (**2**), and (**3**) had higher flux values, probably due to the aqueous solubilities (ranging from 3.32 to 6.56 mg/ml) being higher than 1 mg/ml. (**8**) had an aqueous solubility close to 1 mg/ml (0.76 mg/ml) and a log *D* between 1 and 2 (1.09), which could explain the higher flux obtained. (**6**), (**7**), (**9**), (**10**) and (**11**) had aqueous solubilities far less than 1 mg/ml (ranging from 1.76×10^{-3} to 0.20 mg/ml) and log *D* values between 1 and 2 (ranging from 1.09 to 1.95), leading to lower flux values.

Yano et al. (1986) investigated the skin permeability of eight salicylates, which showed a decrease in absorption with an increase in chain length. A series of investigations have been done on the transdermal delivery of (**1**). The following flux values for (**1**) were obtained: 21.81 ± 3.11 $\mu g/cm^2$ h (Feldmann and Maibach, 1970), and in vivo 24.8 ± 4.4 $\mu g/cm^2$ h and in vitro 29.0 ± 3.1 $\mu g/cm^2$ h (Bronaugh et al., 1982). The previous studies were all performed at neutral pH. In this study the flux for (**1**) was 47.53 ± 5.8 $\mu g/cm^2$ h at pH 4.5, which is a higher flux value than the values obtained in previous studies. Since (**1**) is 99.99% ionised at pH 7.4 and 91.91% ionised at pH 4.5, it makes sense that the flux values obtained in this study was higher, due to the fact that (**1**) is more unionised.

5. Conclusion

The acetylsalicylic acid derivatives were successfully synthesised and the structures were verified by ^{1}H and ^{13}C NMR and MS spectroscopy. The higher flux values of (**1**), (**2**) and (**3**) can be due to the aqueous solubilities being higher than 1 mg/ml. (**8**) had an aqueous solubility of 0.76 mg/ml and a log *D* of 1.09, which could explain the higher flux. The aqueous solubility values of the other acetylsalicylate derivatives were far less than 1 mg/ml, but they had log *D* values between 1–2, which lead to lower flux values, due to the fact that the lipophilic compounds

had difficulty leaving the stratum corneum or for being oils that were found floating on top of the saturated solution. The more lipophilic derivatives may exhibit lower flux values than (**1**), since they are less water soluble. In this study the flux for (**1**) was $47.53 \pm 5.8\ \mu g/cm^2$ h at pH 4.5, which is a higher flux value than those obtained in previous studies, due to the fact that (**1**) is more unionised. The flux of (**1**) is also higher than that of any of the derivatives prepared in this study. Their transdermal application thus has no practical advantage.

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References

Abdou, H.M., 1989. Dissolution, Bioavailability and Bioequivalence. Mack Publishing Company, Easton, Pennsylvania, p. 554.

Barr, M., 1962. Percutaneous absorption. J. Pharm. Sci. 61, 395–409.

Bronaugh, R.L., Stewart, R.F., Congdon, E.R., Giles, A.L., 1982. Methods for in vitro percutaneous absorption studies. I. Comparison with in vivo results. Toxicol. Appl. Pharm. 62, 474–480.

Feldmann, R.J., Maibach, H.I., 1970. Absorption of some organic compounds through the skin in man. J. Invest. Dermatol. 54, 399–404.

Florey, K. (Ed.), 1979. Analytical Profiles of Drug Substances, vol. 8. Academic Press, New York, pp. 3–46.

Giovannucci, E., Egan, K.M., Hunter, D.J., Stamper, M.J., Colditz, G.A., Willett, W.C., Speizer, F.E., 1995. Aspirin and the risk of colorectal cancer in women. N. Engl. J. Med. 333, 609–614.

Hadgraft, J., 1996. Recent developments in topical and transdermal delivery. Eur. J. Drug Metab. Ph. 21, 165–173.

Harrison, E.J., Groundwater, P.W., Brian, K.R., Hadgraft, J., 1996. Azone induced fluidity in human stratum corneum. A Fourier transform infrared spectroscopy investigation using the perdeuterated analogue. J. Control. Release 41, 283–290.

Insel, P.A., 2001. Analgesic-antipyretic and anti-inflammatory agents and drugs employed in the treatment of gout. In: Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, W.R., Gillman, A.G. (Eds.), Goodman & Gilman's The pharmacological Basis of Therapeutics, 10th ed. McGraw-Hill, New York, p. 2148.

Jack, L., Cameron, B.D., Scott, R.C., Hadgraft, J., 1991. In vitro percutaneous absorption of salicylic acid: effect of vehicle pH. In: Scott, R.C., Guy, R.H., Hadgraft, J., Bodde, H.E. (Eds.), Prediction of Percutaneous Penetration. IBC Technical, London, pp. 515–518.

Kligman, A.M., Christophers, E., 1963. Preparation of isolated sheets of human stratum corneum. Arch. Dermatol. 88, 702–705.

Mitragotri, S., 2000. In situ determination of partition and diffusion coefficients in the lipid bilayers of stratum corneum. Pharm. Res. 17, 1026–1029.

Nielsen, N.M., Bundgaard, H., 1989. Evaluation of glycolamide esters and various other esters of aspirin as true aspirin prodrugs. J. Med. Chem. 32, 727–734.

Pefile, S., Smith, E.W., 1997. Transdermal drug delivery: vehicle design and formulation. S. Afr. J. Sci., 147–151.

Rang, H., Dale, M.M., 1999. Pharmacology, fourth ed. Churchill Livingstone, London, p. 830.

Reynolds, J.E.F. (Ed.), 1984. The Martindale: The Extra Pharmacopoeia, 28th ed. The Pharmaceutical Press, London, pp. 235–244.

Roberts, W.J., Sloan, K.B., 2000. Prediction of transdermal flux of prodrugs of 5-fluorouracil, theophylline, and 6-mercaptopurine with a series/parallel model. J. Pharm. Sci. 89, 1415–1431.

Sloan, K.B., 1989. Prodrugs for dermal delivery. Adv. Drug Deliver. Rev. 3, 67–101.

Sloan, K.B., Wasdo, S., Ezike-Mkparu, U., Murray, T., Nickels, D., Singh, S., Shanks, T., Tovar, J., Ulmer, K., Waranis, R., 2003. Topical delivery of 5-fluorouracil and 6-mercaptopurine by their alkylcarbonyloxymethyl prodrugs from water: Vehicle effects on design of prodrugs. Pharm. Res. 20, 639–645.

Winek, C.L., Wahba, W.W., Winek, C.L., Winek Balzer, T., 2001. Avaulable from: https://fscimage.fishersci.com/webimages_FSC/downloads/winek.pdf (date of access: 07 December 2003).

Yano, T., Nakagawa, A., Tsuji, M., Noda, K., 1986. Skin permeability of various non-steroidal anti-inflammatory drugs in man. Life Sci. 39, 1043–1050.